12p 9refs

Presented at the COSPAR

~~Paper for Committee on Space Research~~, Symposium

Warsaw, ~~Poland~~, June 3 - 11, 1963 Submitted for Publication

Tracing [10] conf.

double code
602 1109
2134005

BACTERIA UNDER SIMULATED MARTIAN CONDITIONS

R. S. Young, P. H. Deal, J. Bell, and J. L. Allen [1963]

National Aeronautics and Space Administration
Ames Research Center
Moffett Field, Calif., USA

BACTERIA UNDER SIMULATED MARTIAN CONDITIONS

R. S. Young, P. H. Deal, J. Bell, and J. L. Allen

NASA, Ames Research Center, Moffett Field, Calif., USA

Abstract: The behavior of organisms in simulated Martian conditions is of great importance to Exobiology for two reasons: (1) Because of the extreme environment of Mars, the likelihood of contamination of the planet by earth organisms is considered slight by some scientists. To date, there has been little evidence to contradict this supposition. Such evidence is presented. (2) The selection and adaptation of earth bacteria to Martian conditions is potentially significant in understanding Martian life, if it exists, and may be helpful in designing life-detection techniques and devices. Of course, simulation attempts, based on current knowledge of the Mars environment, may be far from the actual conditions, and extrapolations made from such situations of no real significance. However, generalizations can be made and cautious interpretation of the results of those experiments seems well worth reporting.

A new technique for simulation of known parameters of the Martian environment is discussed along with possible biological implications. The response of bacteria to such simulation is demonstrated in terms of survival and growth, showing that certain bacteria will not only survive, but grow during simulated Martian freeze-thaw cycling if water is present. Ways are demonstrated in which water can be present on Mars although not detectable with current technology. Plans for future experimentation are discussed.

The survival and growth of earth organisms on extraterrestrial bodies (particularly Mars) is of great interest from two points of view. The first is the potential danger of contamination of extraterrestrial bodies by the introduction of earth organisms which may be capable of survival and growth. Such contamination could conceivably obscure or destroy any indigenous life before it is studied or even identified by a life-detection device. Such a loss would be irreversible and could render future reliable study of the biology and biochemistry of the planet impossible. From the point of view of information pertinent to the uniqueness of life on earth and the origins and evolution of life on earth and/or elsewhere in the universe, such a loss would be inexcusable. However, the problem of decontaminating a spacecraft, to prevent such a catastrophe, is enormous, and only if (as has been suggested) earth organisms actually present no hazard to an extraterrestrial body, such as Mars, can the problem be greatly reduced. If, for example, it can be shown that the Martian diurnal freeze-thaw cycle will prevent growth of microorganisms, precautions against contamination could be relaxed somewhat.

The second area of interest in such experimentation is more fundamental in nature - that is, to study the response of familiar organisms to such environmental conditions as exist on Mars and determine rates of selection in, and adaptation to, such environments. To extrapolate from such stimulations in the laboratory to the actual response of Martian organisms to their environment in terms of metabolism and evolution is, strictly speaking, impossible. However, if we assume that Martian life has characteristics which relate it to life on earth or elsewhere in the Universe - for example, a carbon-based chemistry - then such experiments may provide meaningful data from which one can derive generalities about possible life on Mars - its

ecology, metabolism, and detectability. Even here, however, extrapolation is risky, since to simulate the total environment of Mars (even if we knew all its parameters) in the laboratory is impossible with current technology.

2. Methods

It is assumed in these experiments that water is present on Mars, perhaps in some microenvironments, as suggested by Lederberg and Sagan [1], despite the fact that recent observations indicate water in the Martian atmosphere amounting to about 1 percent of the earth's atmospheric water content. A paper is in preparation which describes experiments with a Martian model in which ways can be demonstrated for water to exist on Mars and yet be undetectable by available methods. These experiments were done in a much shortened growing period as compared to those of both Davis [2] and Hawrylewicz [3] so that the temperature cycling more accurately simulated an equatorial Martian summer day-night. The temperature was cycled so that the organisms had only about 4-1/2 hours during which the temperature was above 0° C with a maximum of about 25° C. The organisms spent the remainder of the time in a dry-ice chamber at about -75° C.

Since preliminary experiments indicated no effect of simulated Martian pressures (0.1 atm) on growth of microorganisms, pressure was not controlled in these experiments. The organisms used were Aerobacter aerogenes ATCC No. 129 and an organism tentatively identified as a Pseudomonad collected from the rim of the crater of the volcano Stromboli. Both organisms are facultative anaerobes. A. aerogenes was grown on Difco Heart Infusion broth and the second organism on Difco Brain-Heart Infusion broth. In a typical experiment sterile broth was seeded with a small quantity of medium from an 18- to 22-hour culture

of the given organism grown anaerobically at 25° C. The seeded broth was thoroughly mixed and distributed in 1.5 ml quantities to a number of Pyrex glass tubes. The tubes were sealed under nitrogen at 1 atmosphere by drawing out in a flame. A viability assay was made in duplicate on each of two tubes immediately after sealing by preparing serial dilutions in broth and plating on solid agar. Agar plates were incubated aerobically at 25° C and colony counts were made the following day. The remaining tubes were placed in a dry-ice chamber at -75° C. On the following day, and each day thereafter for the duration of the experiment, the tubes were removed from the dry-ice chamber and thawed at 25° C in an incubator. Thawing required approximately 30 minutes, after which viability assays (in duplicate on each of two tubes) were made at intervals over the next 4-1/2 hours; the remaining tubes were then returned to the dry-ice chamber. Control experiments were run using the same technique but without any intervals of freezing.

3. Results

Fig. 1 > Fig. 1 shows the results of a typical experiment with A. aerogenes. Each data point on the graph represents an average of four plate counts of two individual experimental tubes. The growing time of the experimental tubes is shown to be approximately 4-1/2 hours per day, and the rate of killing due to night-time freezing can be determined. It can be seen that the experimental organisms grow very satisfactorily under these conditions with a fairly constant rate of killing due to freezing. These experiments were repeated several times under identical conditions always with the same result.

Fig. 2 > Fig. 2 shows the results of a typical experiment with the organism tentatively identified as Pseudomonas sp. As can be seen, the results are

essentially the same as with A. aerogenes. It is of interest to note in these experiments that there appears to be no significant lag phase after the commencement of growth at the beginning of each day-time period. The only lag phase to be seen is at the initiation of the experiment. It is also of interest to note that these two organisms are not unique in this response, for there appear to be others, including yeast, which grow under these conditions. These experiments indicate then the ability of certain earth organisms not only to survive, but to grow satisfactorily under simulated Martian conditions given a suitable medium, including water. The data show a generation time in the experimental bacteria which approaches that of the controls during the growing period of 4-1/2 hours, so that there is at least a 4000-fold increase in viable experimental organisms during the 3-1/2 to 4 days of experiment, discounting the number of organisms killed by freezing and thawing. The completely lethal effect of repeated freeze-thawing of bacteria and other organisms to be found in the literature [4 and 5] is not seen in these experiments because of the fact that sufficient time is provided for the organisms to reproduce in satisfactory numbers to maintain a more than adequate viability of the colony. Preliminary experiments on other bacteria, including one which may be a "variant" in the A. aerogenes culture, show no killing at all due to freeze-thaw cycling under these conditions. On the other hand, some organisms (Spirillum itersonii, Rhodopseudomonas gelatinoso, Pseudomonas ovalis) tested are killed quickly. Experiments are now under way to determine the shortest period of growth time and the minimum nutritional requirement for such organisms to maintain themselves under these conditions.

Experiments are also being performed to determine the minimum water requirement for earth organisms under simulated Martian conditions. If it

can be shown that earth organisms are able to survive and grow under the most rigorous simulation of Martian conditions possible in the laboratory, this should lend considerable emphasis to the need for space vehicle sterilization as well as contribute information to a program aimed at the detection of life on the planet Mars. At the same time, a great deal can be, and is being learned concerning the adaptability of life, as we know it on earth, to environmental extremes and perhaps on the mechanisms of adaptation, selection, and evolution of organisms on earth or elsewhere in the universe. Much has been said in recent years concerning the possibility of life on the planet Mars [6 and 7], and the significance of simulation experiments such as those described in this paper have been widely discussed. Perhaps many biologists, who have devoted considerable time and attention to the possibility of Martian life, have become somewhat emotionally involved. This has, in many cases, led to extremes in extrapolating what little data there actually are which are pertinent to the possibility of life. For example - the phenomenon of the "wave of darkening" which proceeds in a seasonal fashion from pole through equator of Mars has been interpreted by some to indicate a springlike process attributable to a living phenomenon [8]. The change in color proceeds at a relatively constant rate of about 45 kilometers per day [9] from the edge of the Martian pole cap through the equator into the opposite hemisphere during a Martian spring. It has been said that this color change is due to the rapid growth of organisms of one kind or another caused by the sudden availability of water at the Martian surface during the seasonal progression; however, the astronomical data indicate that in northern latitudes during this progressive color change, the actual surface temperatures never rise above zero, and it is only at the Martian equator and a few degrees north

and south of the equator during the Martian summer that the temperatures ever rise above zero and even then only for a few hours during the day. It seems extremely unlikely that Martian life, if it is at all similar to life on earth, could have evolved the capability of reproduction in this rapid a fashion in latitudes of the planet where the temperature never at any time rises above the freezing point and, in some cases, where the temperature never rises as high as -20° C.

As mentioned earlier in this paper, experiments are under way to determine the actual tolerances which can be induced in earth organisms which may possibly cast some light on this problem. Obviously, any conclusions concerning the possible biology of Mars must be made and interpreted with great caution. Experiments of the type described in this paper point out the need to maintain at least an open mind on the possibility of Martian (or extraterrestrial) life, by demonstrating the incredible adaptability of our own microorganisms to environments approaching that of Mars.

REFERENCES

1. J. Lederberg, and C. Sagan, Microenvironments for Life on Mars. Proc. Nat. Acad. Sci. 48 (1962) pp. 1473-1475

2. I. Davis, and J. D. Fulton, Microbiologic Studies on Ecologic Considerations of the Martian Environment. Review 2-60, School of Aviation Medicine, Brooks AFB, Texas (1959)

3. E. Hawrylewicz, B. Gowdy, and R. Ehrlich, Micro-organisms under a Simulated Martian Environment. Nature 193 (1962) p. 497

4. A. P. Harrison, Jr., Survival of Bacteria upon Repeated Freezing and Thawing. J. Bact. 70 (1955) pp. 711-715

5. A. U. Smith, Biological Effects of Freezing and Supercooling. Williams and Wilkins Co. (1961)

6. G. deVaucouleurs, Physics of the Planet Mars, Faber and Faber (1954)

7. F. Salisbury, Martian Biology. Science 136 (1962) pp. 17-26

8. A. Dollfus, In Planets and Satellites, G. P. Kuiper, ed., Univ. of Chicago Press, Chicago (1961)

9. A. G. Wilson, The Atmospheres of Mars and Venus, by Kellogg, W. W. and C. Sagan, Ed. Publ. No. 944, Nat. Acad. of Sci., Washington, D. C. (1961)

FIGURE CAPTIONS

Fig. 1: Aerobacter aerogenes.

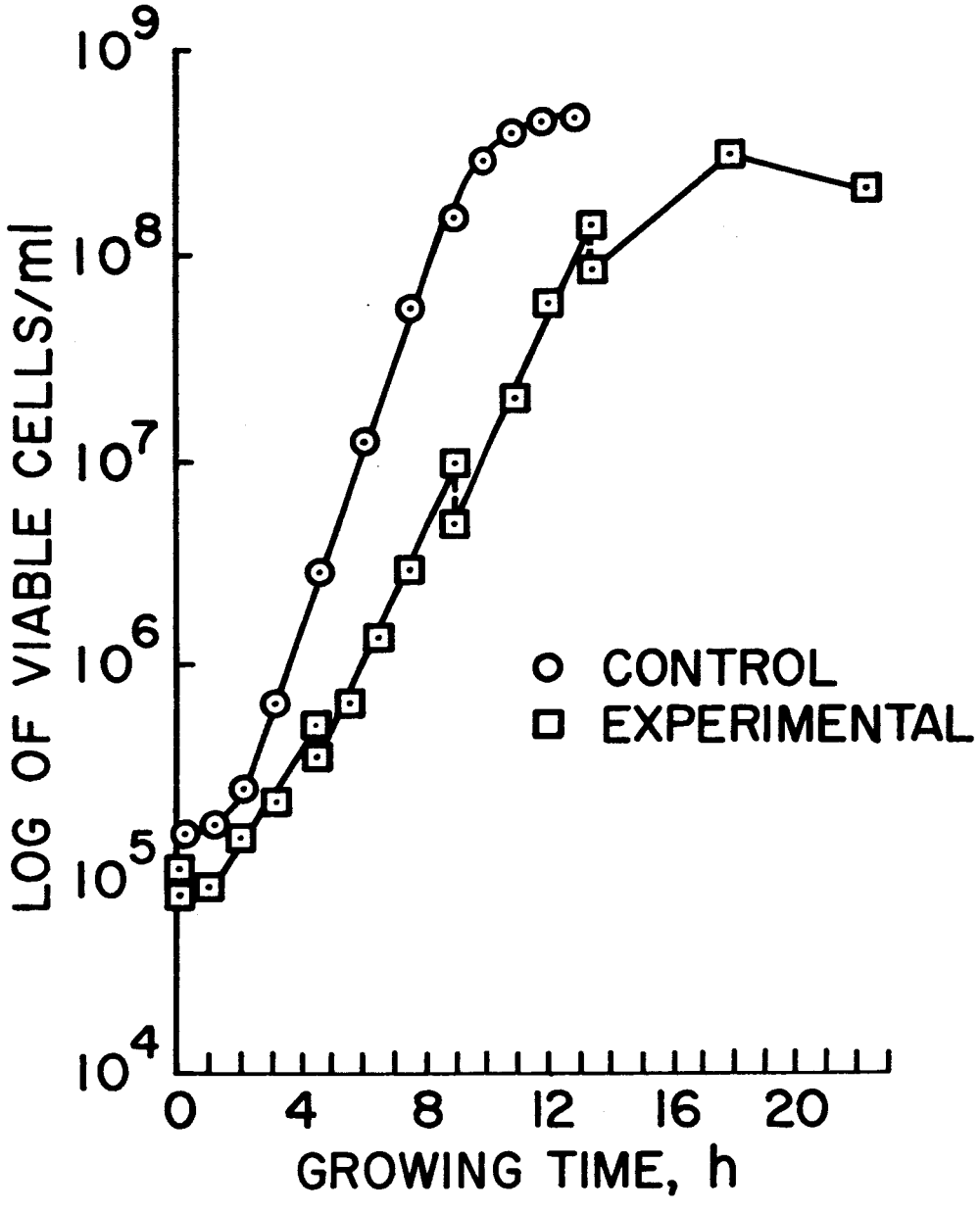
Control: Uninterrupted growth.

Experimental: Growth interrupted by freezing. Vertical discontinuity illustrates decline in numbers due to death as a result of freeze-thaw process.

Fig. 2: Pseudomonas sp.

Control: Uninterrupted growth.

Experimental: Growth interrupted by freezing. Vertical discontinuity illustrates decline in numbers due to death as a result of freeze-thaw process.



A-30732-1

Fig. 1: Aerobacter aerogenes
Control: Uninterrupted growth.
Experimental: Growth interrupted by freezing. Vertical discontinuity illustrates decline in numbers due to death as a result of Freeze-Thaw process.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
AMES RESEARCH CENTER, MOFFETT FIELD, CALIFORNIA

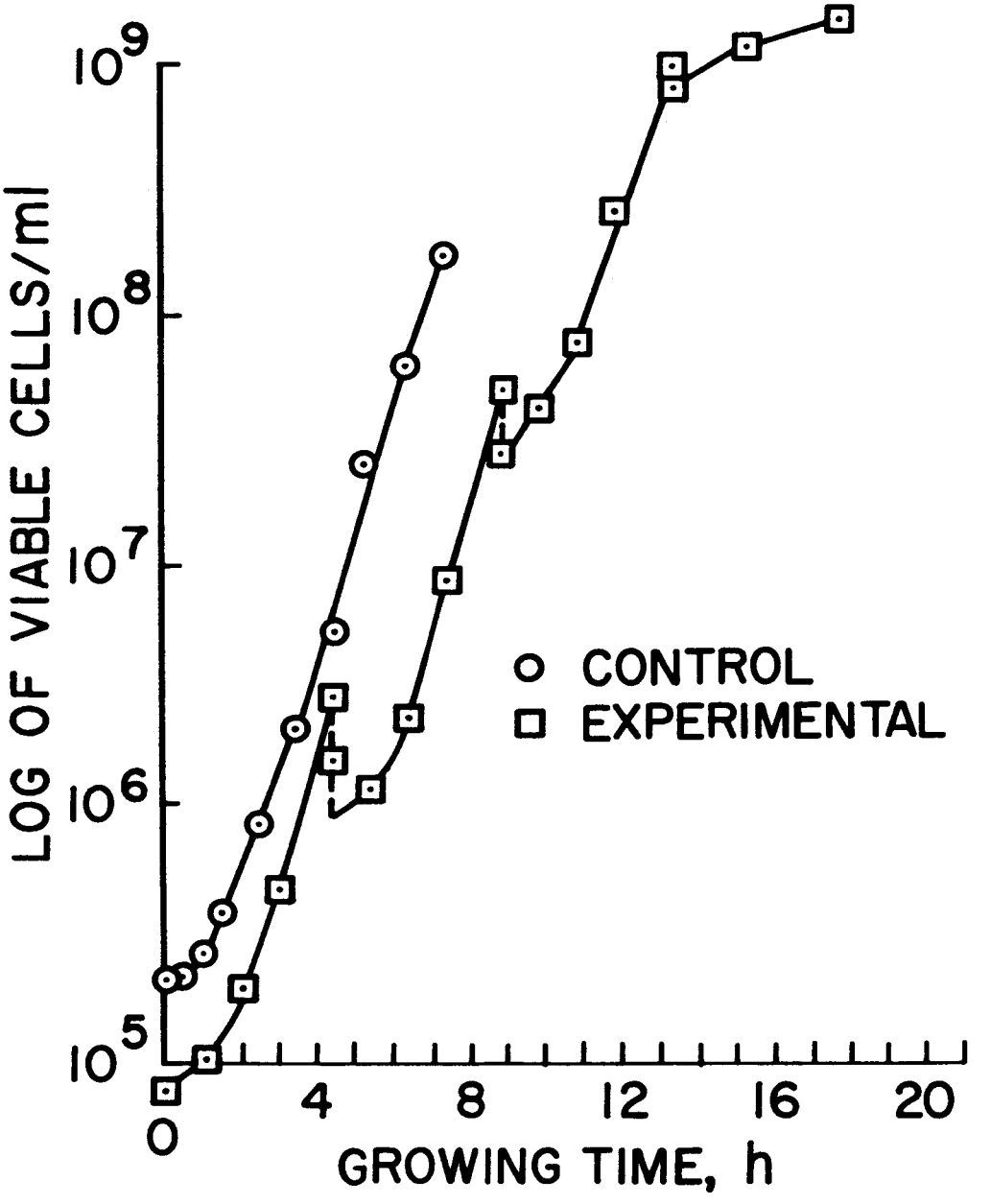


Fig. 2: Pseudomonas sp.
Control: Uninterrupted growth.
Experimental: Growth interrupted by freezing.
Vertical discontinuity illustrates decline in numbers due to death as a result of Freeze-Thaw process.

A-30732-2

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
AMES RESEARCH CENTER, MOFFETT FIELD, CALIFORNIA